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Clinical update of Ad-p53 gene therapy for lung cancer

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Lung cancer has become the most common cause of cancer-related mortality in the world in both men and women. Despite the development of aggressive protocols utilizing chemotherapy, radiation therapy, and surgery, the overall survival of lung cancer has changed little, with less than 15% of patients surviving longer than 5 years. Because of these poor results and the recognition that cancer is caused by molecular defects, research has focused on developing novel therapeutics to try to correct these underlying genetic defects and reverse the malignant phenotype.

The development of viral vectors (adenoviral and retroviral) has allowed this strategy to be carried out in the clinic because these vectors have allowed genes to be transferred into tumors at high levels of efficiency and expression. This paper focuses on preliminary results from clinical studies utilizing p53 gene therapy strategies in lung cancer to determine the therapeutic potential of this novel therapy and to try to identify subsets of patients who could potentially benefit from this treatment.

Molecular role of p53 in lung cancer

The p53 gene is the most commonly mutated gene in lung cancer [1,2]. The gene is mutated in 50% to 70% of patients who have lung cancer. Additionally, in a large proportion of cases in which there is no mutation, p53 is inactivated through binding by high levels of Mdm-2 protein or it is functionally inactive because downstream genes such as the pro-apoptotic Bcl-2 family members (which p53 transactivates) are mutated or transcriptionally inactive [3]. The function of the p53 gene is not completely understood, but

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its protein product can function as a transcription factor that regulates critical genes involved in DNA repair, cell cycle control, and apoptosis induction [4]. The gene is often described as the "guardian of the genome" because of its known role in safeguarding genomic integrity against mutational changes. In response to DNA-damaging agents or ionizing radiation, p53 protein levels rise in the cell. This leads to upregulation of p21 and DNA repair genes that lead to G1 cell cycle arrest and upregulation of DNA repair genes, which try to repair the DNA damage. If repair is not possible, the cell is induced to undergo apoptosis by upregulation of another set of p53-regulated genes, which leads to cellular apoptosis. In many cases of neoplasia, cancer cells have evaded apoptosis despite severely mutated DNA because of p53 defects. Replacement of wt-p53 by gene transfer techniques then allows restoration of the apoptotic process and destruction of the tumor cells. In many cases cancer cells—unlike normal cells—can be primed for self-destruction when wt-p53 is restored because of their abnormal genomes.

Because the wt-p53 gene transactivates and controls multiple other genes involved in growth regulation, the gene transfer of a single functional p53 gene has the potential to affect and correct multiple other genetic defects critical to the malignant phenotype. Additionally, because normal cells already contain wt-p53 and normal genomes, the gene transfer of wt-p53 genes does not induce cellular apoptosis and allows a therapeutic treatment targeted only at the cancer cells. Unlike conventional agents such as radiation therapy or chemotherapy in which normal cells are also affected, wt-p53 gene transfer does not appear to induce significant toxicity to normal cells.

The optimal system to transfer the wt-p53 gene into tumor cells has not yet been determined and is a subject of ongoing research. The most common gene transfer system utilized in lung cancer trials involves viral systems utilizing retroviruses or adenoviruses. These viral vectors allow transduction of tumors with the wt-p53 gene through intratumoral injections or bronchoalveolar lavage. Recombinant adenoviruses (Ad-p53) have several advantages over other retroviruses for clinical trials because they can be grown easily in large quantities and are able to transduce both dividing and non-dividing cells [5,6]. Because of these advantages, adenoviral vectors have become the most common viral vector system utilized in lung cancer gene therapy trials.

Anti-tumor activity after p53 gene transfer has been demonstrated in pre-clinical studies in vitro and in vivo, in both immunodeficient and immunocompetent animal models. Fujiwara and colleagues [7] demonstrated a therapeutic effect in an orthotopic lung cancer model transduced with a retroviral wild-type p53 expression vector. Zhang [8] achieved high-level gene transfer and expression using an adenoviral p53 (Ad-p53) construct with growth inhibition of human lung cancer cells bearing multiple genetic defects. Ad-p53 gene transfer also induced significant "bystander" killing, in which wt-p53–transduced cells mediated killing of surrounding cells that had not themselves been transduced, possibly by mechanisms involving

inhibition of angiogenesis [9–11], immune upregulation [12–14], or secretion of soluble Fas protein [15]. These pre-clinical studies with viral vectors formed the groundwork for the subsequent human clinical gene therapy trials in lung cancer (Table 1).

Clinical trials with p53 gene transfer alone in lung cancer

The first clinical trial involving transfer of wt-p53 into lung cancer used a retroviral vector containing a wt-p53 cDNA [16]. In this trial, performed by Roth et al at the University of Texas M. D. Anderson Cancer Center, retroviral wt-p53 was injected directly into endobronchial tumors under bronchoscopic guidance in four patients and CT guidance in five patients [16]. The presence of wt-p53 was demonstrated in the biopsied tumor specimens of three patients. Three of seven evaluable patients showed evidence of tumor regression. Furthermore, there was no significant toxicity even with repeated injections. This preliminary trial demonstrated the feasibility and safety of gene therapy strategies based on the restoration of wt-p53 gene function in advanced non–small-cell lung cancer. Future clinical trials did not use retroviral vectors, however, because large scale clinical trials were limited by difficulties in generating high retroviral titers. Because of these limitations, adenoviral vectors, which could be produced at substantially higher viral titers ($>10^{12}$ vp [viral particles]) and were able to infect replicating and non-replicating cells, were used in subsequent clinical trials (Table 1).

Schuler et al [17] performed a Phase I study in 15 patients who had locoregionally advanced or metastatic Non-small cell lung cancer (NSCLC) in Europe (Table 1). A single intratumoral injection of Ad-p53 (SCH 58500) was delivered by bronchoscopy or CT guidance. Doses were begun at 7.5×10^{10} vp and were escalated to 7.5×10^{12} vp. No clinically significant toxicity was observed, and successful transfer of wild-type p53 was demonstrated in post-treatment biopsies of six patients. Transient local disease control was observed in four of the six patients who had demonstrated wild-type p53 gene transfer. All six patients demonstrated progression of distant lesions that had not been injected, suggesting that the clinical effect of Ad-p53 was limited to the locoregional injected site (Table 2). Additionally, in the five patients who were evaluable and did not have demonstrated p53 gene transfer, only two stabilized and three progressed, suggesting an association with gene transfer and efficacy. Vector-related toxicity was minimal and was predominantly limited to Grade I and II fevers and chills and procedure-associated pain. This study confirmed that intratumoral injection of Ad-p53 (SCH 58500) was feasible and resulted in successful gene transfer at higher doses of Ad-p53. Perhaps because only one injection was performed, clinical responses were sustained for only short periods.

In another Phase I study in patients who had metastatic NSCLC, Swisher and Roth et al evaluated 28 patients whose tumors had failed all conventional treatments, including chemotherapy, radiation therapy, and surgery

Table 1
Ad-p53 gene therapy trials in non-small cell lung cancer

Gene therapy trials	Regimen	No. of patients	Mode of delivery	Toxicity	Biological activity	Clinical activity
P53 alone—NSCLC						
Roth et al, 1996 [16]	Retroviral-p53 alone	9	IT	Minimal	Yes	Yes
Swisher et al, 1999 [18]	Ad-p53[c] alone	40	IT	Minimal	Yes	Yes
Schuler et al, 1998 [25][a]	Ad-p53[d] alone	15	IT	Minimal	Yes	Yes
P53 alone—Broncheoalveolar						
Kubba et al, 2000 [20]	Ad-p53[c] alone	26	BAL	Some Gr III dyspnea	Yes	Possible[b]
P53 and chemotherapy—NSCLC						
Nemunaitis et al, 1999 [24]	Ad-p53[c] + Cis	24	IT	Minimal	Yes	Yes
Schuler et al, 2001 [17][a]	Ad-p53[d] + Pac–Carbo Or	13	IT	Minimal	Not evaluated	Possible[b]
	Ad-p53[d] + Cis–V	12	IT			
P53 and radiation therapy—NSCLC						
Swisher et al, 2000 [28,29]	Ad-p53[c] + 60 Gy Radiation therapy	19	IT	Minimal	Yes	Suggested

Abbreviations: BAL, bronchoalveolar lavage; Carbo, carboplatin; Cis, cisplatinum; IT, intratumoral, Pac, paclitaxel; V, vinorelbine.

[a] Each patient had two comparable lesions (one lesion was treated with Ad-p53 and one lesion was not treated with Ad-p53).

[b] No statistical difference in radiologic response but tendency for increased tumor shrinkage by measurement in lesion injected with Ad-p53.

[c] Ad-p53 vector: INGN 201-Introgen Therapeutics.

[d] Ad-p53 vector: SCH 58500-Schering Plough.

Table 2
Ad-p53 trials with comparison lesions (one injected and one observed)

Clinical trial	Schuler et al, 1998 [25]	Kubba et al, 2000 [20]	Schuler et al, 2001 [17]
Treatment regimen	Ad-p53[c] alone	Ad-p53[d] alone	Ad-p53[c] + Pac/ Carbo or Cis/V
Mode of delivery	IT	BAL	IT
Radiologic response[a,b] (injected/non-injected lesion)			
Partial response	0/0	1(4%)/0	2(8%)/1(4%)
Stabilization	7(47%)/1(7%)	13(57%)/8(35%)	11(44%)/11(44%)
Progression	6(40%)/11(73%)	9(39%)/15(65%)	11(44%)/10(40%)
Not evaluated	2(13%)/2(13%)	0/0	1(4%)/3(12%)
Total patients	15	23	25
Size response[a,b] (injected/non-injected lesion)	ND	ND	(−62%)/(−35%)

Abbreviations: BAL, bronchoalveolar lavage; Carbo, carboplatin; Cis, cisplatinum; IT, intratumoral, ND, not determined; Pac, paclitaxel; V, vinorelbine.

[a] Each patient had 2 comparable lesions (one lesion was treated with Ad-p53 and one lesion was not treated with Ad-p53).

[b] No statistical difference in radiologic response but tendency for increased tumor shrinkage by measurement in lesion injected with Ad-p53.

[c] Ad-p53 vector: INGN 201-Introgen Therapeutics.

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(Table 1) [18]. Patients were treated by direct intratumoral injection of Ad-p53 (INGN 201) by way of bronchoscopy or CT guidance on a monthly basis for up to 6 months unless progression or toxicity was noted. Doses were begun at 10^7 vp and escalated to 10^{12} vp. This study demonstrated that multiple doses of Ad-p53 (INGN 201) could be safely administered to patients who had advanced metastatic NSCLC. Eighty-four doses were administered to 28 patients with 56 of the doses repeated injections for periods of up to 6 months. Even though neutralizing anti-adenovirus antibody levels rose dramatically after the first dose [14,19], vector-related adverse events were minimal. Transient Grade I or II fevers and chills were the most common vector-associated events, and they lasted 12 to 48 hours. Toxicity was so low that the last ten patients were treated as outpatients without difficulty. The second important observation of this trial was that intratumoral delivery of Ad-p53 (INGN 201) resulted in wt-p53 gene transfer in the injected tumor even in the face of neutralizing levels of anti-adenovirus antibody. Vector-specific primers that incorporated flanking regions of the adenovirus were used to ensure detection of adenovirally transferred p53 mRNA rather than native p53. Gene transfer appeared to be dose dependent and could still be seen even with neutralizing anti-adenovirus antibodies present in the circulation. The third important finding of this study was that locoregional antitumoral activity was noted following Ad-p53 gene transfer. Two of 28 (7%) evaluable patients demonstrated a partial response and

16/28 evaluable patients (57%) demonstrated stabilization of disease for periods of 2 to 24 months. The study also demonstrated that delivery of vector was feasible in a large scale clinical trial.

Kubba and colleagues have reported preliminary results in a Phase I trial in which Ad-p53 (INGN 201) was administered by bronchial lavage to patients with bronchoalveolar lung cancer [20]. This unusual variant of lung cancer often presents with a diffuse intra-alveolar presentation that is not amenable to surgery and is often resistant to chemotherapy. Tumor cells tend to grow in a thin layer along the alveoli. They are not accessible to direct injection but are approachable by bronchial lavage. This study was initiated through ECOG (E6597) and was performed primarily at Vanderbilt Cancer Center and the University of Wisconsin. Ad-p53 was administered by bronchoalveolar lavage (BAL) for two cycles with biopsies 3 days after administration. Doses were started at 2×10^9 vp and were escalated to 2×10^{12} vp. Perhaps because of the delivery system and inflammatory responses to the vector, Grade III toxicity was noted in five of 16 patients and consisted primarily of hypoxia and dyspnea. Dose-limiting toxicities were seen in two of six patients at 2×10^{12} vp but only one out of 11 patients at lower doses. Pathological responses were noted in the treated lobe of two of the first five patients, and four of the first nine patients demonstrated improvements in their Diffusion Lung Capacity Oxygen (DLCO) of 20% or more. These responses were associated with symptomatic improvement in four of the first 11 patients, suggesting that even minor responses in this disease might result in symptomatic improvement. Preliminary results from this study suggest that BAL administration of Ad-p53 is feasible and might provide a novel approach to a disease that is resistant to most conventional therapies. Phase II studies are being proposed to assess the clinical benefit of combining Ad-p53 with chemotherapy in bronchoalveolar carcinoma becuase chemotherapy alone has traditionally been ineffective in this disease. These trials will also need to identify the factors associated with airway toxicity so that optimal delivery of Ad-p53 can be obtained in the future with minimal treatment-related morbidity.

Clinical trials with p53 gene transfer and chemotherapy in lung cancer

Inactivation of the p53 gene by missense mutations or deletions has been reported to enhance cellular resistance to chemotherapeutic agents [4]. Preclinical studies have demonstrated that gene transfer of Ad-p53 in combination with chemotherapy can reverse chemoresistance and lead to synergistic antitumoral effects in animal models [21–23]. Because of these observations several clinical trials have evaluated the effect of combining chemotherapy and Ad-p53 in lung cancer.

Nemunaitis et al evaluated in a Phase I study the effect of Ad-p53 (INGN 201) in combination with cisplatin [24]. Twenty-four patients who had metastatic NSCLC and had failed all conventional therapies (including

cisplatinum-containing regimens in >40%) received monthly intratumoral injections up to 6 months if no progression developed with cisplatinum (80 mg/m^2) 3 days before. As with the Swisher et al study, doses were escalated from 10^7 vp to 10^{12} vp in cohorts of three [18]. Toxicity was minimal, with the most common adverse event attributable to Ad-p53 being Grade I or II fevers and chills. Chemotherapy-associated toxicity did not appear to be increased by the concomitant administration of Ad-p53. This interesting observation suggests that conventional agents can be given with Ad-p53 without significantly increasing toxicity. Clinical response at the injected tumor demonstrated partial response in two patients (8%), stabilization of disease in 17 patients (70%) and progression in four (28%); one patient was unevaluable because of progressive disease in distant non-injected sites. Both patients achieving partial responses had previously progressed on prior platinum-based chemotherapy regimens. This study suggested that antitumor strategies combining Ad-p53 (INGN 201) with chemotherapy were feasible and potentially beneficial. This strategy would allow improved locoregional control with intratumoral Ad-p53 and systemic treatment with chemotherapy.

Schuler et al performed a Phase II study (Tables 1, 2) in metastatic NSCLC evaluating patients who had two comparable lesions. All patients received chemotherapy either three cycles of carboplatin (AUC 6) plus paclitaxel (175 mg/m^2) or cisplatin (100 mg/m^2) plus vinorelbine (25 mg/m^2). Additionally, Ad-p53 was injected directly into one lesion and the other lesion was used as a control and was not injected. The authors hoped to demonstrate an enhanced radiologic response in the injected lesion compared with the non-injected lesion. Injected tumors received a dose of Ad-p53 (SCH 58500) of 7.5×10^{12} vp on the first day of each cycle (three total cycles) [25]. As with the Nemunaitis et al study [24], Ad-p53 (SCH 58500) resulted in minimal vector-related toxicity and no increase in chemotherapy related adverse events. Overall radiologic response rates as determined by NCI criteria were not significantly different, however, although only 25 patients were entered on two different regimens. Additionally, mean local tumor regression as measured by size was greater in the Ad-p53 injected lesion compared to the control lesion, especially with the cisplatin and vinorelbine regimen. The implications of this trial are (1) the combination of Ad-p53 and chemotherapy agents does not significantly increase overall toxicity, and (2) the observation that Ad-p53 can safely be delivered with platinum-based chemotherapy regimens is confirmed. Although clear improvements in injected tumor responses could not be demonstrated using classic radiographic response criteria, there did appear to be a trend towards enhanced tumor shrinkage when size criteria were used (Table 2). Further follow-up will be needed to determine if these size differences lead to prolonged time to progression. This study emphasizes the need for a randomized study to be performed to determine clinical efficacy especially because these results are less encouraging than the author's initial Phase I study (Table 2) [17].

Clinical trials with p53 gene transfer and radiation therapy in lung cancer

Pre-clinical studies have also suggested that p53 mutations in tumors increase resistance to ionizing radiation [26]. Adenoviral-mediated wt-p53 gene transfer has been shown to radiosensitize resistant tumors without affecting normal fibroblasts [10]. Additionally, animal models have demonstrated a synergistic antitumoral effect when external beam radiation therapy is combined with Ad-p53 [27] Fig. 1. Because of these observations and the poor locoregional control of lung cancers treated with radiation or chemoradiation, a Phase II study was designed by Swisher and Roth et al to assess the locoregional anti-tumoral activity of Ad-p53 and radiation therapy in patients with non-metastatic NSCLC [28,29] Fig. 2. This study was composed primarily of early stage NSCLC patients who were not physiologically capable of undergoing surgery or more locoregionally advanced (Stage IIIA /IIIB) NSCLC patients who could not tolerate chemoradiation because of associated comorbidities. The study carefully assessed response at the primary injected site by utilizing pathologic negative biopsies 3 months after completion of Ad-p53 and radiation therapy as an endpoint rather than radiologic responses. This endpoint was used to avoid the inherent difficulties of trying to assess pathologic response radiologically and to provide a better assessment of tumor viability becuase size criteria often do not reflect biologic status [30]. LeChevalier et al [31] performed a study with radiation and chemoradiation that demonstrated that the pathologic negative biopsy rate was a critical predictor of long-term cure. This study demonstrated that only 20% of patients treated with radiation alone demonstrated biopsy-negative tumors 3 months after radiation therapy. The proposed Phase II gene therapy trial sought to see a doubling of the pathologic negative biopsy rate from 20% to 40% and estimated that 49 patients would be needed to demonstrate this increase. The Phase II gene therapy

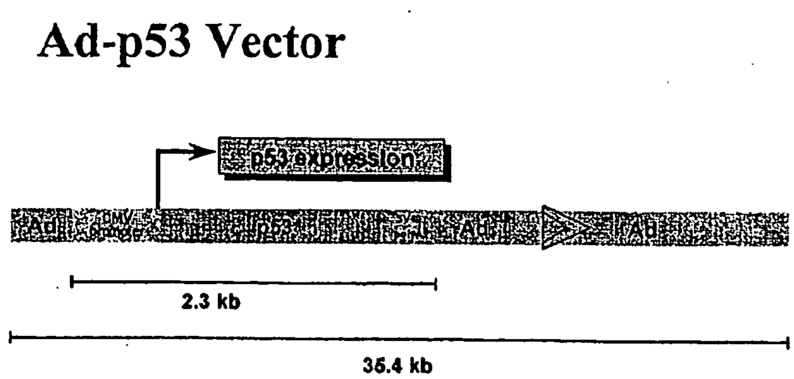

Fig. 1. Diagram of first generation replication incompetent adenoviral p53 vector (INGN 201-Introgen Therapeutics or SCH58500-Schering Plough) utilized in Ad-p53 gene therapy trials in lung cancer.

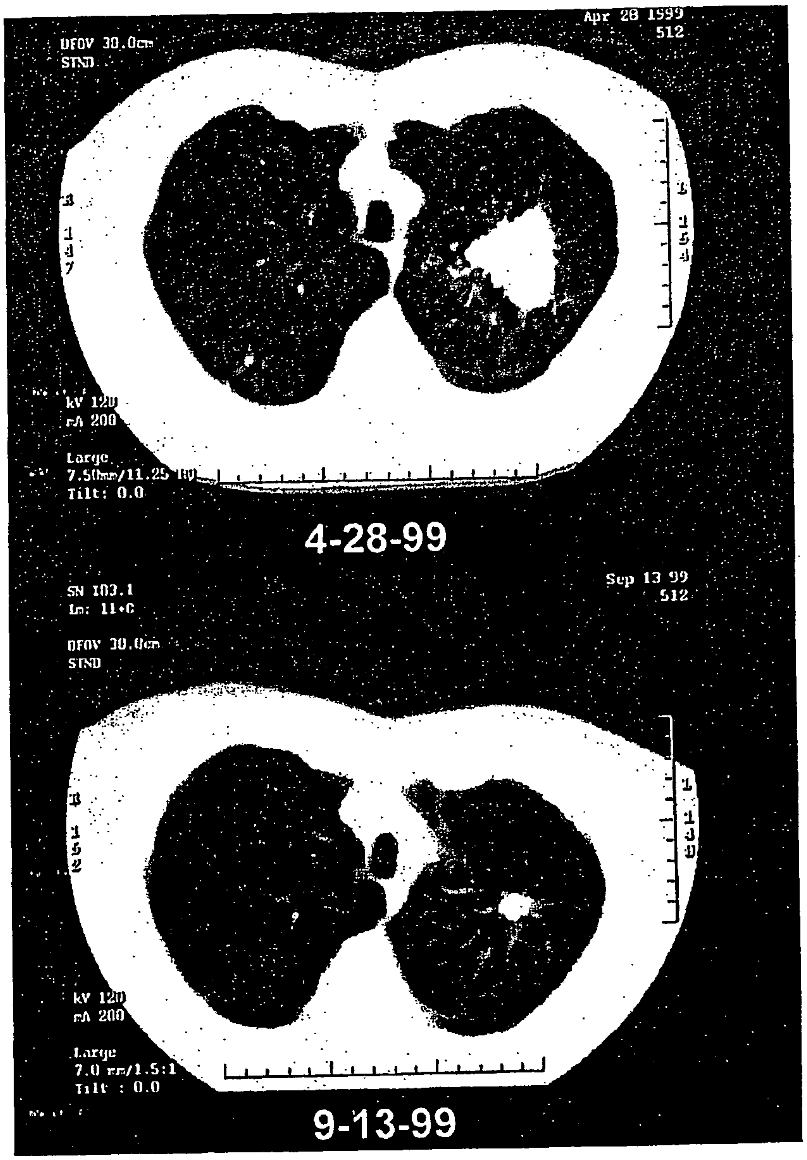

Fig. 2. Patient with left upper lobe tumor treated on Phase II trial (Swisher/Roth [28]) with three intratumoral instillations of Ad-p53 and external beam radiation therapy (60 Gy). CT scans are prior to treatment and 3 months after completion of therapy. Core biopsy 3 months after treatment demonstrated no residual cancer and the patient remains disease-free 36 months after treatment.

trial was designed to inject Ad-p53 (INGN 201) on days one, 18, and 32 of radiation therapy (60 Gy; Table 1). Ad-p53 (INGN 201) doses were escalated from 3×10^{11} vp to 3×10^{12} vp in the first nine patients. When safety was established, the remaining patients were injected directly into the primary tumor at 3×10^{12} vp of Ad-p53 by way of bronchoscopy or CT guidance. The first clinical observation of the study was that toxicity was minimal and the combined regimen could be delivered successfully in an outpatient setting. Sixty-one CT-guided biopsies and injections were performed in the first 13 CT injected patients with 13 pneumothoraces. These pneumothoraces were managed with observation (eight patients) or percutaneous pleural catheter (five patients) as outpatients without the need for hospital admission. No treatment-related mortality was observed and radiation therapy toxicity did not appear to be increased. Pathologically negative biopsies were noted in eight out of 11 biopsied patients, suggesting a high pathologic control rate at the primary tumor. The 1-year overall survival rate was 45.5% with most failures occurring because of metastatic progression rather than local failure [28]. This study suggests that Ad-p53 (INGN 201) can be given in conjunction with radiation therapy in a group of high-risk patients with low toxicity in an outpatient setting. The high negative pathologic control rate observed in this study was encouraging, but the continued metastatic failure emphasized the need to combine Ad-p53 (INGN 201) in the future with chemotherapy agents to try to address the distant disease which was presumably not being addressed by Ad-p53 and radiation therapy. These results emphasize the need for a multi-institutional, randomized Phase III trial to determine the therapeutic potential of Ad-p53 and chemoradiation to treat locoregionally advanced NSCLC.

Future Ad-p53 gene therapy trials

The clear benefit of intratumoral Ad-p53 compared to conventional agents is the low toxicity, which might be due to the therapeutic selectivity of wt-p53. Additionally, except perhaps in the patients with endobronchial tumors, Ad-p53 alone does not appear adequate to lead to long-term lung cancer cure. The low toxicity of Ad-p53 suggests, however, that strategies of combination with conventional agents might increase antitumoral efficacy without increasing treatment-related morbidity.

The second important observation of these trials is that the majority of the effect of Ad-p53 appears limited to the locoregional area where high concentrations of vector allow high levels of gene transduction (Table 2). This observation suggests that therapeutic strategies and trials must be designed with an understanding and expectation of primarily locoregional effect. Given these observations, several clinical subsets emerge in NSCLC that might benefit from improved Ad-p53–mediated locoregional control (Table 3). Clearly, early stage patients who cannot tolerate surgery because of poor pulmonary function or other comorbidities and would be treated

Table 3
Ad-p53 gene therapy: clinical subsets to target for trials

1. Early stage lung cancer (Stages I and II)
Unresectable because of comorbidities:
Ad-p53 intratumoral + radiation therapy
2. Locoregionally advanced lung cancer (Stage III)
Unresectable because of extent of disease or comorbidities:
Ad-p53 intratumoral + concurrent chemoradiation if good performance (proposed Phase II/III multi-institutional trial-Swisher/Roth and colleagues)
Ad-p53 intratumoral + sequential chemoradiation if lower performance
3. Metastatic lung cancer (Stage IV)
Endobronchial disease symptomatic:
External Beam RT + Ad-p53 intratumoral
Brachytherapy RT + Ad-p53 intratumoral (proposed Phase II multi-institutional trial-Komaki and colleagues)
Ad-p53 alone
Symptomatic primary or metastatic lesion
Palliative RT (30 Gy) + Ad-p53 intratumoral (ongoing ECOG trial—Kubba/Choy and colleagues)
Unresectable broncheoalveolar carcinoma
Chemotherapy + Ad-p53 bronchial lavage (proposed Phase II E5599 multi-institutional trial, Kubba and colleagues)

with radiation therapy alone represent a subset that would benefit from the addition of Ad-p53 to radiation therapy. Efforts to improve locoregional control with increased radiation doses alone have been limited by increased toxicity, and the potential exists for improved radiation sensitivity with the addition of Ad-p53 without increased toxicity. Other potential clinical subsets include metastatic NSCLC patients who have endobronchial disease that could be treated with Ad-p53 alone, Ad-p53 and external beam radiation therapy, or Ad-p53 and brachytherapy. This traditionally difficult group of patients appears to respond favorably to Ad-p53 [32]. Another group of patients who have metastatic NSCLC are patients with symptomatic primary or distant tumors that would be treated with palliative radiation therapy (30 Gy) alone. Currently, ECOG is evaluating the feasibility of Ad-p53 (INGN 201) in combination with 30 Gy of radiation therapy for recurrent or previously radiated NSCLC. This trial, initiated by Kubba, Choy, and Schiller, will assess feasibility, locoregional control, and biologic parameters.

The role of Ad-p53 in locally advanced NSCLC (Stage III) still needs to be evaluated further. There are several randomized studies that demonstrate that improved survival in Stage III NSCLC can be achieved with improved locoregional control [33–36] even though micro-metastatic disease is often present. Recent studies have suggested that concurrent chemoradiation can lead to enhanced survival compared to sequential chemoradiation, but it is limited to high performance status patients because of increased toxicity with concurrent delivery. The low toxicity profile of Ad-p53 might allow

combinations with concurrent or sequential chemoradiation to further improve locoregional control and long-term survival benefits without increasing toxicity. To test this idea, the authors have proposed a Phase II/III trial to evaluate this concept in a randomized fashion. The study proposes to compare concurrent chemoradiation alone (Taxol and Carboplatin and 66 Gy RT) with concurrent chemoradiation and Ad-p53 delivered. The study is designed in a Phase II/III Bayesian fashion to allow early analysis in the Phase II portion of locoregional pathologic control and efficacy. If there is no clear evidence of improved locoregional control in the Phase II portion, additional patients would not have to be entered in the Phase III portion because long-term improvements in survival would be unlikely in such a situation. The trial proposes to include a 3-month pathologic biopsy to better determine locoregional control and compare the experimental arm with Ad-p53 and concurrent chemoradiation with concurrent chemoradiation alone. This trial would allow a determination of the role of Ad-p53 in locoregionally advanced NSCLC in a randomized, multi-institutional setting.

Future avenues of research

These preliminary clinical trials have also demonstrated several areas on which future translational gene therapy research should focus. First, although intratumoral delivery of Ad-p53 can transduce injected tumors with a high level of efficiency, non-injected or distant tumors do not appear to be affected. Because most patients who have NSCLC have metastatic or micro-metastatic disease at presentation, local injections of Ad-p53 do not address all of the disease. Attempts to deliver adenoviral vectors intravenously are limited by high levels of circulating anti-adenoviral antibodies, which develop and preclude redelivery intravascularly. Recently, however, Templeton et al [37] reported development of an improved extruded cationic liposome formulation that significantly increased systemic gene delivery. These findings have led to successful regression of tumors in animal models with intravenous delivery of liposome-p53 DNA complexes as reported by Ramesh et al [38]. If these findings can be translated into the clinic with intravenous delivery of liposomal gene complexes, gene transfer could take the next step and start to address the large number of patients who have systemic metastases who are currently only palliated and not cured with conventional chemotherapy.

These clinical trials demonstrated that some—but not all—of the injected tumors responded to Ad-p53. Additionally, some of the tumors that initially responded to Ad-p53 ultimately failed, suggesting the development of resistance. Studies in the authors' laboratory have suggested that wt-p53 induces apoptosis in lung cancer cells, in part by upregulating expression of the pro-apoptotic genes Bak and Bax, which then mediate cytochrome c release [39] from the mitochondria with subsequent caspase activation and apoptosis

induction. These observations led the authors to develop an adenoviral vector that overexpressed the pro-apoptotic Bak or Bax gene in an effort to bypass p53-resistant tumors [40–44]. Further research must focus on developing methods to ensure that the increased tumor killing with these vectors is specific for tumors to prevent non-specific toxicity to normal cells [45]. These preliminary efforts give hope for the future that novel laboratory efforts based on preliminary gene therapy clinical trial results will lead ultimately to more effective and long-lasting treatments for NSCLC.

Summary

These preliminary Phase I and II gene therapy trials in NSCLC have demonstrated that Ad-p53 gene transfer is associated with low toxicity and evidence of antitumoral activity at the locoregional site. Efforts to enhance antitumoral efficacy with chemotherapy and radiation therapy have not increased Ad-p53 toxicity and appear to be feasible. Randomized Phase III studies are now needed to determine the potential of Ad-p53 to improve overall survival in selected subsets of NSCLC patients. Future gene therapy research is required to develop systemic delivery systems and to overcome p53 tumor resistance. It is hoped that these efforts will ultimately lead to a novel mode of therapy to complement conventional chemotherapy, radiation therapy, and surgical treatment strategies.

References

[1] Fisher MD. Strategies to restore p53 function in patients with lung cancer. Clin Lung Cancer 2001;3:99–101.

[2] Mukhopadhyay T, Maxwell SA, Roth JA. p53 suppressor gene. Austin, TX: RG Landes; 1995.

[3] Rampino N, Yamamoto H, Ionov Y, et al. Somatic frameshift mutations in the Bax gene in colon cancers of the microsatellite mutator phenotype. Science 1997;275:967–9.

[4] Lowe SW, Ruley HE, Jacks T, et al. p53 dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993;74:957–67.

[5] Roth JA, Cristiano RJ. Gene therapy for cancer: what have we done and where are we going? J Natl Cancer Inst 1997;89:21–39.

[6] Dameron KM, Volpert OV, Tainsky MA, et al. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 1994;265:1582–4.

[7] Fujiwara T, Cai DW, Georges RN, et al. Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. J Natl Cancer Inst 1994;86:1458–62.

[8] Zhang WW, Fang X, Mazur W, et al. High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus. Cancer Gene Ther 1994;1:5–13.

[9] El-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817–25.

[10] Kawabe S, Munshi A, Zumstein LA, et al. Adenovirus-mediated wild-type p53 gene expression radiosensitizes non-small cell lung cancer cells but not normal lung fibroblasts. Int J Radiat Biol 2001;77:185–94.

[11] Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of human bax gene. Cell 1995;80:293–9.
[12] Carroll JL, Nielsen LL, Pruett SB, et al. The role of natural killer cells in adenovirus-mediated p53 gene therapy. Mol Cancer Ther 2001;1:49–60.
[13] Molinier-Frenkel V, le Boulaire C, le Gal F-A. Longitudinal follow-up of cellular and humoral immunity induced by recombinant adenovirus-mediated gene therapy in cancer patients. Hum Gene Ther 2000;11:1911–20.
[14] Yen N, Ioannides CG, Xu K, et al. Cellular and humeral immune responses to adenovirus and p53 protein antigens in patients following intratumor injection of an adenovirus vector expressing wild-type p53 (Ad-p53). Cancer Gene Ther 2000;7:530–6.
[15] Owen-Schaub LB, Zhang W, Cusack JC, et al. Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol Cell Biol 1995;15:3032–40.
[16] Roth JA, Nguyen D, Lawrence DD, et al. Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nat Med 1996;2:985–91.
[17] Schuler M, Herrmann R, DeGreve JL, et al. Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small cell lung cancer; results of a multicenter phase II study. J Clin Oncol 2001;19:1750–8.
[18] Swisher SG, Roth JA, Nemunaitis J, et al. Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. J Natl Cancer Inst 1999;91:763–71.
[19] Roth JA, Swisher SG, Merritt JA, et al. Gene therapy for non-small cell lung cancer: a preliminary report of a phase I trial of adenoviral p53 gene replacement. Semin Oncol 1998; 25:33–7.
[20] Kubba S, Adak S, Schiller J, et al. Phase I trial of adenovirus p53 in bronchioloalveolar cell lung carcinoma (BAC) administered by bronchoalveolar lavage. Proc Am Soc Clin Oncol 2000;19:487a.
[21] Fujiwara T, Grimm EA, Mukhopadhyay T, et al. Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res 1994;54:2287–91.
[22] Inoue A, Narumi K, Matsubara N, et al. Administration of wild-type p53 adenoviral vector synergistically enhances the cytotoxicity of anti-cancer drugs in human lung cancer cells irrespective of the status of p53 gene. Cancer Lett 2000;157:105–12.
[23] Nguyen DM, Spitz FR, Yen N, et al. Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. J Thorac Cardiovasc Surg 1996;112:1372–7.
[24] Nemunaitis J, Swisher SG, Timmons T, et al. Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. J Clin Oncol 2000;18:609–22.
[25] Schuler M, Rochlitz C, Horowitz JA, et al. A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer. Hum Gene Ther 1998;9:2075–82.
[26] Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiation. Proc Natl Acad Sci USA 1993;90:5742–6.
[27] Spitz FR, Nguyen D, Skibber JM, et al. Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation. Clin Cancer Res 1996;2: 1665–71.
[28] Swisher SG, Roth JA, Komaki R, et al. A phase II trial of adenoviral mediated p53 gene transfer (RPR/INGN 201) in conjunction with radiation therapy in patients with localized non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2000;19:461a.
[29] Swisher SG, Roth JA, Komaki R. Induction of pro-apoptotic mediators and tumor regression following intratumoral delivery of adenoviral p53 (RPR/INGN 201) and radiation therapy in patients with non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2000;20:257a.

[30] Rusch VW, Giroux DJ, Kraut MJ, et al. Induction chemoradiation and surgical resection for non-small cell lung carcinomas of the superior sulcus: initial results of southwest oncology group trial 9416 (intergroup trial 0160). J Thorac Cardiovasc Surg 2001;121:472–83.

[31] Le Chevalier T, Arriagada R, Quoix E, et al. Radiotherapy alone versus combined chemotherapy and radiotherapy in nonresectable non-small-cell lung cancer; first analysis of a randomized trial in 353 patients. J Natl Cancer Inst 1991;83:417–23.

[32] Weill D, Mack M, Roth J, et al. Adenoviral-mediated p53 gene transfer to non-small cell lung cancer through endobronchial injection. Chest 2000;118:966–70.

[33] Curran WJ, Scott C, Langer C, et al. Phase III comparison of sequential versus concurrent chemoradiation for PTS with unresected stage III non-small cell lung cancer (NSCKC): initial report of radiation therapy oncology group 9RTOG) 9410. Proc Am Soc Clin Oncol 2000;19:484a.

[34] Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. J Clin Oncol 1999;17:2692–9.

[35] Saunders M, Dische BA, Harvey A, et al. Continuous hyperfractionated accelerated radiotherapy (CHART) versus conventional radiotherapy in non-small-cell lung cancer: a randomised multicentre trial. Lancet 1997;350:161–5.

[36] Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small cell lung cancer. N Engl J Med 1992;326:524–30.

[37] Templeton NS, Lasic DD, Frederik PM, et al. Improved DNA:liposome complexes for increased systemic delivery and gene expression. Nat Biotechnol 1997;15:647–52.

[38] Ramesh F, Saeki T, Templeton NS, et al. Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppression genes using an improved liposome vector. Mol Ther 2001;3:337–50.

[39] Pearson AS, Spitz FR, Swisher SG, et al. Up-regulation of the pro-apoptotic mediators bax and bak after adenovirus-mediated p53 gene transfer in lung cancer cells. Clin Cancer Res 2000;6:887–90.

[40] Kawaga S, Gu J, Honda T, et al. Deficiency of caspase-3 in MCF-7 cells blocked bax-mediated nuclear fragmentation but not cell death. Clin Cancer Res 2001;7:1474–80.

[41] Kawaga S, Gu J, Swisher SG, et al. Antitumor effect of adenovirus-mediated bax gene transfer on p53 sensitive and p53-resistant cancer lies. Cancer Res 2000;60:1157–61.

[42] Kawaga S, Pearson SA, Ji L, et al. A binary adenoviral vector system for expressing high levels of the pro-apoptotic gene bax. Gene Ther 2000;7:75–9.

[43] Pataer A, Fang B, Yu R, et al. Adenoviral bak overexpression mediated caspase-dependent. Cancer Res 2000;60:788–92.

[44] Pataer A, Smythe WR, Yu R, et al. Adenovirus-mediated bak gene transfer induces apoptosis in mesothelioma cell lines. J Thorac Cardiovasc Surg 2001;121:61–7.

[45] Gu J, Sagawa S, Takakura M, et al. Tumor-specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the bax gene to cancers. Cancer Res 2000;60:5359–64.